

## EFFECT OF SALICYLIC ACID APPLIED THROUGH ROOTING MEDIUM ON DROUGHT TOLERANCE OF WHEAT

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### Abstract

An experiment was conducted to assess whether exogenously applied SA through the rooting medium could mitigate the adverse effects of water stress on plant growth, photosynthesis and nutrient status of two wheat genotypes. For this purpose, salicylic acid @ 0, 5, and 10 mg L<sup>-1</sup> was applied through the rooting medium to plants of two wheat lines growing in plastic beakers (250 mL) filled with Hoagland's nutrient solution containing 0 or 19% PEG<sub>8000</sub> to represent two water regimes of control (0 MPa) and -0.6 MPa respectively. Different levels of SA applied through the rooting medium increased photosynthetic rate in both cultivars under non-stress conditions but only in S-24 under water stress conditions. Exogenous application of 5 or 10 mg L<sup>-1</sup> SA caused an increase in stomatal conductance, transpiration rate, and sub-stomatal of water stressed plants of cv. S-24 whereas it was true for droughted plants of MH-97 only when 5 mg L<sup>-1</sup> SA applied. Cultivar S-24 was generally higher in N and P contents of shoot and root than that in genotype MH-97 under both normal and water stress conditions. A decrease in shoot and root N contents of both genotypes and shoot and root P contents of genotype S-24 only was observed in stressed plants when 5 mg L<sup>-1</sup> of SA was applied through the rooting medium, whereas the same was true for root P and shoot Ca<sup>2+</sup> contents in the non-stressed plants of both cultivars. Application of salicylic acid through the rooting medium significantly reduced the root K<sup>+</sup> of two cultivars under both normal and water deficit conditions. Although, exogenously applied SA through the rooting medium had growth promoting effects under non-stress conditions, it did not mitigate the adverse effects of drought stress on growth of both cultivars, though genotype MH-97 showed some recovery under water stress conditions.

### Introduction

Limited water supply is a major problem to wheat production worldwide. According to an estimate, in developing countries, about 45% of the 120 million ha sown to wheat are prone to drought (Rajaram, 2001). Although selection and breeding is the ultimate way to produce stress tolerant crop plants, exogenous application of osmoprotectants, growth promoters and antioxidant compounds to plants has been considered a short-term solution to alleviate the adverse effects of different stresses on plants during the last decade (Khan *et al.*, 2003; Iqbal & Ashraf, 2005; Raza *et al.*, 2006; Arfan *et al.*, 2007). However, any compound can be exogenously applied either as a pre-sowing seed treatment, as a foliar spray or through the rooting medium (Iqbal & Ashraf, 2005; Ashraf & Foolad, 2005, 2007).

Salicylic acid (SA) is an endogenous regulator of phenolic nature, which is involved in the regulation of various physiological processes in plants (Shakirova *et al.*, 2003). For example, it improves plant growth (Metwally *et al.*, 2003; Khodary, 2004), transpiration rates, stomatal regulation and photosynthesis (Khan *et al.*, 2003), ion uptake and transport (Gunes *et al.*, 2005). During the last 20 years it aroused the interest of many plant scientists

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due to its ability to induce systemic acquired resistance (SAR) in plants against different pathogens, which is manifested in the appearance of pathogenesis related proteins (PR), while SA is considered to serve as a signal molecule in the induction of expression of these genes (Sakhabutdinova *et al.*, 2003). Many studies have so far been conducted aiming at identifying its role in abiotic stress tolerance in different crops. Results of these studies suggest that exogenously applied SA can induce salt tolerance (Shakirova *et al.*, 2003) and drought resistance in wheat (Singh & Usha, 2003), resistance to heavy metals stress in rice (Mishra & Choudhuri, 1999), NaCl and osmotic stress tolerance in *Arabidopsis* seedlings (Borsani *et al.*, 2001), and multiple stress tolerance in bean and tomato (Senaratna *et al.*, 2000).

Many studies on drought tolerance of wheat have been carried out, but the basic research on biochemical and physiological roles of exogenously applied salicylic acid through the rooting medium on drought tolerance of wheat is scarce. Thus, the present study was conducted to examine whether and how exogenously applied salicylic acid through the rooting medium ameliorates the adverse effects of drought on various growth and photosynthetic attributes and mineral status of this crop.

### Materials and Methods

To study the effect of salicylic acid (SA) applied through the rooting medium on plants of two wheat genotypes, S-24 and MH-97, growing under control or water deficit conditions was carried out in plastic beakers (250 mL) filled with Hoagland's nutrient solution containing different concentrations of PEG<sub>8000</sub> (0 and 19%) and SA (0, 5 and 10 mg L<sup>-1</sup>). The experiment was conducted in the wirehouse of the Department of Botany, University of Agriculture, Faisalabad, during the year 2004-2005.

The experiment was laid out in a completely randomized design with four replications. A total of 48 plastic beakers were divided into two groups of 24 beakers each representing a specific water regime. Each group was further divided into two subgroups of 12 plastic beakers each for the two wheat genotypes, S-24 and MH-97. In order to create two water regimes of control and -0.6 MPa, solutions of 0 and 19% polyethylene glycol (PEG<sub>8000</sub>) in half strength Hoagland's nutrient solution were prepared. The seeds of the two wheat genotype were surface sterilized with 10% Sodium hypochlorite solution for five minutes and washed three times with distilled water. Twenty-five seeds of each wheat genotypes were germinated in Petri dishes each containing 5 mL Hoagland's nutrient solution containing different concentrations of PEG<sub>8000</sub> (0 and 19%) and SA (0, 5 and 10 mg L<sup>-1</sup>) in a growth room.

The six germinated seeds from each Petri dish were then transferred onto a painted iron gauze whose lower surface was made to just touch with 250 mL solution of different concentrations of PEG<sub>8000</sub> (0 and 19%) and SA (0, 5 and 10 mg L<sup>-1</sup>) in half strength Hoagland's nutrient solution contained in each plastic beaker. The painted iron gauzes were used for allowing the roots of germinating seedlings to pass through their pores. The level of treatment solution in each plastic beaker was maintained by adding distilled water on daily basis, if desired. For application of SA through the rooting medium 0, 1.66, or 3.33 mL of stock solution containing 75 mg SA (Sigma Aldrich, Japan) in 100 mL of distilled water were added to each of the 48 plastic beakers after drawing out the same quantities of the half strength Hoagland's nutrient solutions of different concentrations of PEG<sub>8000</sub> (0 and 19%) from the plastic beakers for attaining 0, 5 and 10

mg L<sup>-1</sup> levels of SA respectively. Forty five days after the germination of seeds plants of two wheat genotypes were harvested and data on various morphological characteristics like length of shoots and roots and fresh and dry masses of shoots and roots were recorded. In addition to the morphological characteristics, data on the following gas exchange parameters and mineral elements were also recorded.

**Gas exchange parameters:** Before harvest measurements of CO<sub>2</sub> assimilation rate (*A*), transpiration rate (*E*), stomatal conductance (*g<sub>s</sub>*) and sub-stomatal CO<sub>2</sub> concentration (*C<sub>i</sub>*) were made on a fully expanded second leaf of each plant 6 weeks after the germination of seeds using an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England). Measurements were performed from 10.00 to 13.00 hours by making adjustments like molar flow of air per unit leaf area 403.3 mmol m<sup>-2</sup> s<sup>-1</sup>, atmospheric pressure 99.9 kPa, water vapor pressure into chamber ranged from 6.0 to 8.9 mbar, PAR at leaf surface was maximum upto 1711 μmol m<sup>-2</sup> s<sup>-1</sup>, temperature of leaf ranged from 28.4 to 32.4°C, ambient temperature ranged from 22.4 to 27.9°C and ambient CO<sub>2</sub> concentration was 352 μmol mol<sup>-1</sup>.

**Determination of mineral elements:** The oven dried ground materials (0.1 g) of shoot and root of each treatment were digested with 2 mL of sulfuric acid – hydrogen peroxide mixture according to the method of Wolf (1982). The digested extract was filtered and used for the determination of N, P, K<sup>+</sup> and Ca<sup>2+</sup>. The potassium (K<sup>+</sup>) and calcium (Ca<sup>2+</sup>) contents were determined by flame photometer (Jenway PFP 7). Nitrogen was estimated by micro-Kjeldhal's method (Bremner, 1965). Phosphorus (P) was determined spectrophotometrically following Jackson (1958).

**Statistical analysis:** Analysis of variance of the data for each attribute was computed using the MSTAT-C Computer Program (MSTAT Development Team, 1989). The Duncan's New Multiple Range Test at 5% level of probability was used to test the differences among mean values (Steel & Torrie, 1980).

## Results

Polyethylene glycol (PEG<sub>8000</sub>) induced water stress caused a significant reduction in fresh and dry masses of shoots and roots, and length of shoot while it caused a non-significant increase in root length of cv. S-24 (Fig. 1). Exogenous application of 5 mg L<sup>-1</sup> of SA through the rooting medium significantly increased fresh and dry masses of shoots and roots of both cultivars under control conditions, whereas the same concentration reduced shoot fresh and dry weights of cv. S-24 under water deficit conditions. Under water stressed conditions, cv. MH-97 responded positively to supply of SA through the rooting medium by showing increases in fresh and dry masses of shoots and roots and shoot length. Shoot length of both cultivars under non-stressed condition and that of cv. MH-97 under water stressed conditions showed a slight increase due to application of SA through the rooting medium.

Water deficit conditions reduced CO<sub>2</sub> assimilation rate, stomatal conductance, transpiration rate and sub-stomatal CO<sub>2</sub> concentration in both cultivars. However, cultivars did not differ significantly in these gas exchange attributes except in substomatal CO<sub>2</sub> concentration. Different levels of SA applied through the rooting medium caused a significant increase in net CO<sub>2</sub> assimilation rate in both cultivars under

non-stress conditions, but only in S-24 under water stress conditions (Fig. 2). Due to application of 5 mg L<sup>-1</sup> SA through the rooting medium a slight improvement in stomatal conductance was observed in S-24 under control conditions and in MH-97 under drought conditions. Similarly, transpiration rate was also increased in cv. MH-97 due to 5 mg L<sup>-1</sup> SA application through the rooting medium under both control and water stress conditions, whereas the same was true in cv. S-24 when 10 mg L<sup>-1</sup> SA was applied. Moreover, a slight increase in sub-stomatal CO<sub>2</sub> concentration was observed in stressed plants of MH-97 when 5 mg L<sup>-1</sup> SA was applied through the rooting medium. Neither the drought treatments nor the different levels of SA applied through the rooting medium have any significant effect on the water use efficiencies of the two cultivars.

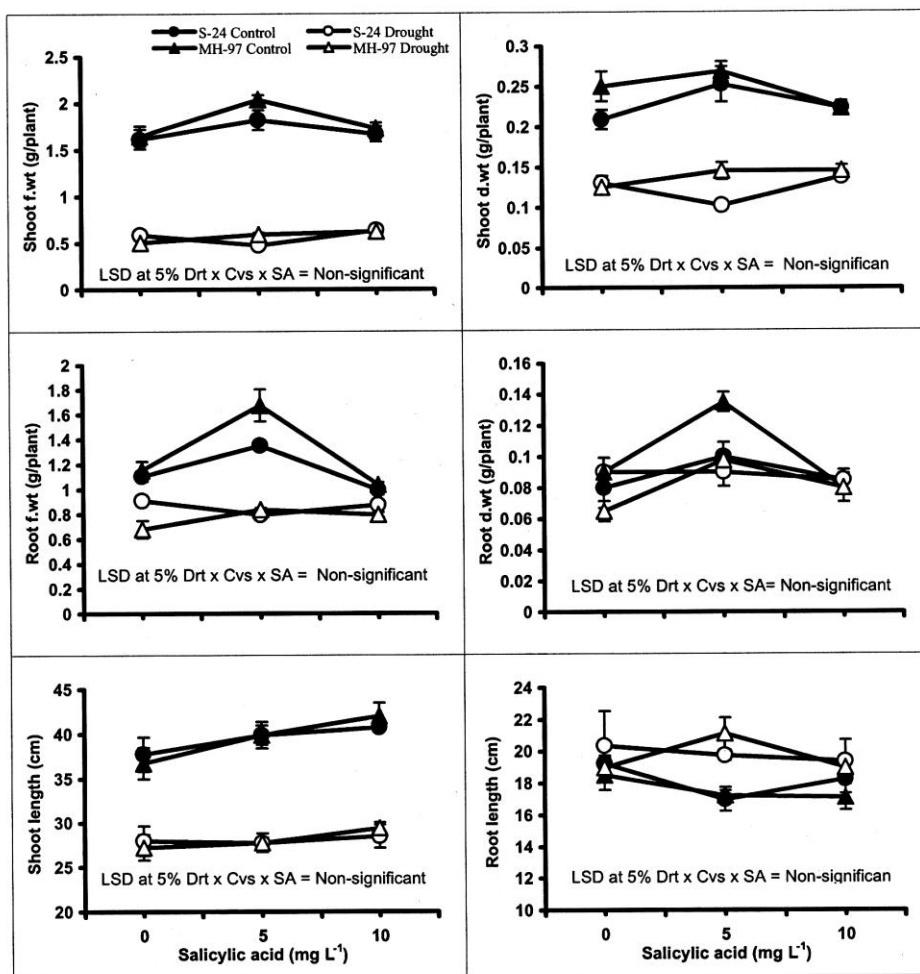


Fig. 1. Fresh and dry masses of shoots and roots, and shoot and root lengths of plants of two spring wheat lines when different levels (0, 5 or 10 mg L<sup>-1</sup>) of salicylic acid (SA) were exogenously applied through the rooting medium containing 0% or 19% PEG<sub>8000</sub> (= -0.6 MPa) for 45 days in hydroponics.

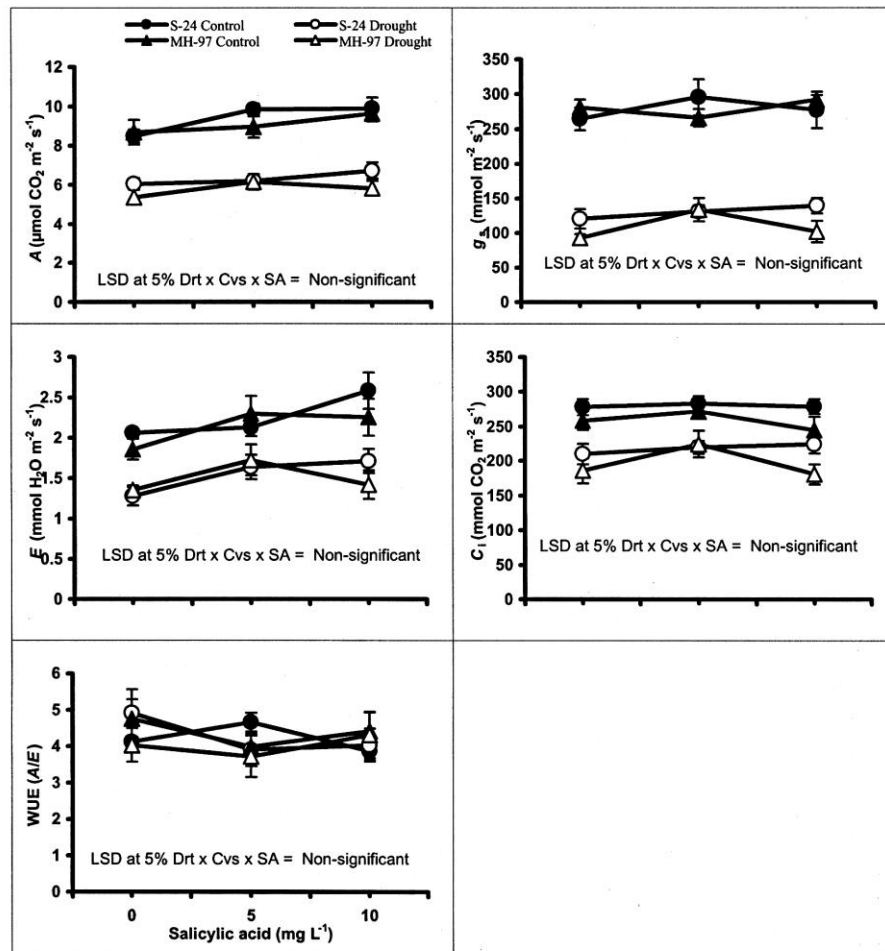


Fig. 2. Net CO<sub>2</sub> assimilation rate (A), stomatal conductance ( $g_s$ ) sub stomatal CO<sub>2</sub> concentration ( $C_i$ ), transpiration rate (E) and water use efficiency (WUE) of plants of two spring wheat lines when different levels (0, 5 or 10 mg L<sup>-1</sup>) of salicylic acid (SA) were exogenously applied through the rooting medium containing 0% or 19% PEG<sub>8000</sub> (= -0.6 MPa) for 45 days in hydroponics.

Drought stress significantly reduced the N, P, K<sup>+</sup> and Ca<sup>2+</sup> contents of shoots and roots of both cultivars. Cultivar S-24 was generally higher in N and P contents of shoot and root than that in cv. MH-97 under both normal and water stress conditions. Exogenous application of SA did not significantly affect the shoot N of both cultivars under normal conditions, whereas under water deficit conditions it caused reduction in shoot N in both cultivars. A maximum reduction in shoot N was observed in stressed plants of both cultivars when 5 mg L<sup>-1</sup> of SA was applied through the rooting medium (Fig. 3). In contrast, exogenous application of SA through the rooting medium did not significantly alter the root N of both cultivars, under normal or water deficit conditions. Although exogenous application of SA did not change the shoot and root P content significantly, it caused a reduction in shoot and root P of stressed plants of both cultivars, whereas the same was true for root P in non-stressed plants of both cultivars.

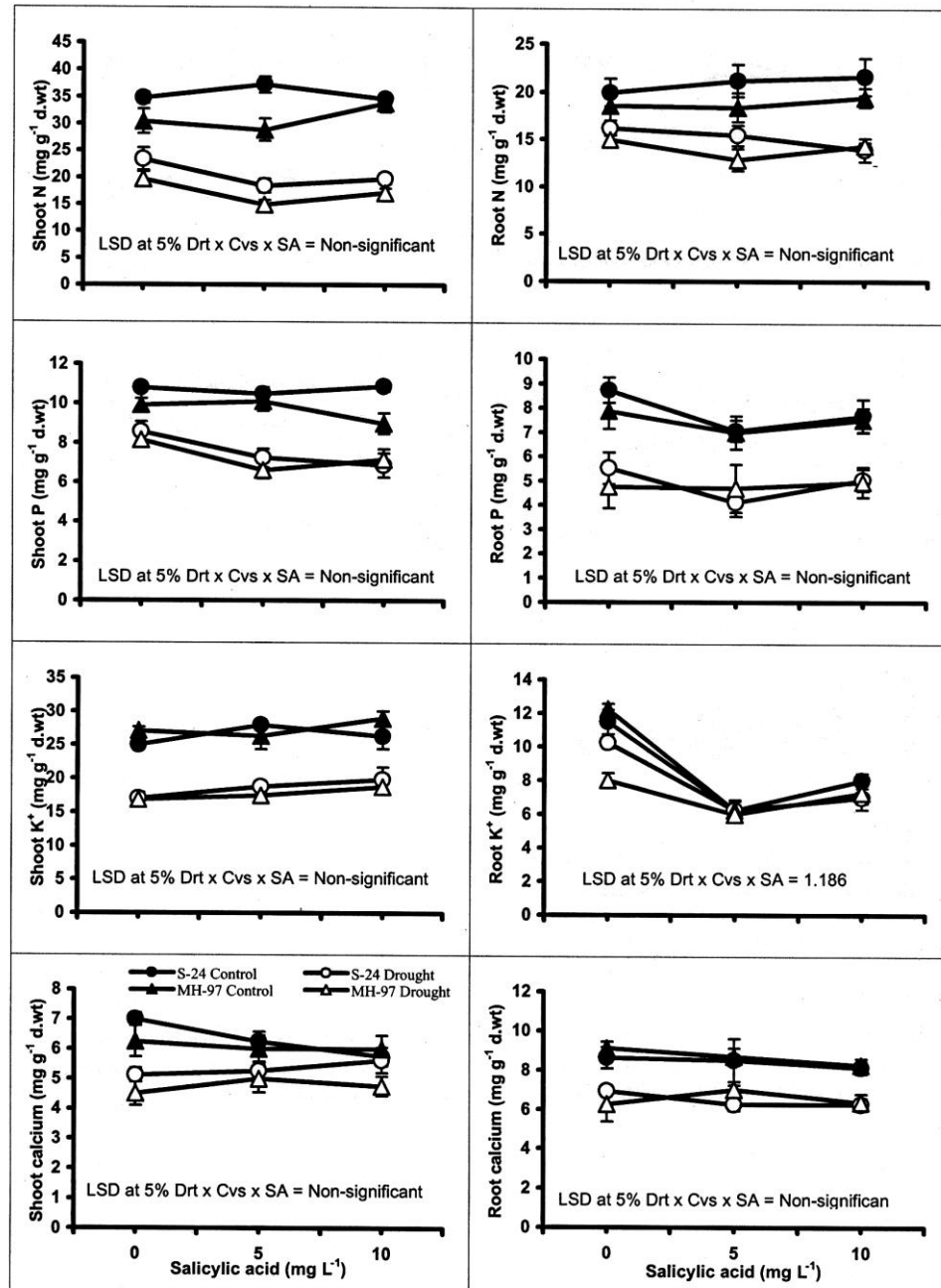


Fig. 3. Shoot and root N, P, K<sup>+</sup> and Ca<sup>2+</sup> of plants of two spring wheat lines when different levels (0, 5 or 10 mg L<sup>-1</sup>) of salicylic acid (SA) were exogenously applied through the rooting medium containing 0% or 19% PEG<sub>8000</sub> (= -0.6 Mpa) for 45 days in hydroponics.

Cultivar S-24 showed higher root  $K^+$  than cv. MH-97 under water deficit conditions. Exogenous application of SA did not change the shoot  $K^+$  of both cultivars under normal or water stress conditions, but in contrast application of salicylic acid through the rooting medium significantly reduced the root  $K^+$  of two cultivars under both normal and water deficit conditions. Similarly, shoot and root  $Ca^{2+}$  of both cultivars was not changed due to different levels of SA applied through the rooting medium under either moisture regime.

## Discussion

Results of the experiments showed that water stress caused a marked reduction in growth of both wheat cultivars. The two cultivars did not differ much in their growth patterns under both control and water deficit conditions. In some previous studies, it was found that cv. S-24 is a salt tolerant (Pritchard *et al.*, 2001) and cv. MH-97 moderately salt sensitive (Iqbal & Ashraf, 2005). It is well established that drought and salt stress tolerance share the osmotic component. However, non-significant differences between the cultivars under non-stressed and water stress conditions indicated that salt tolerance of cv. S-24 was mainly due to tolerance to ionic component of salt stress.

Although exogenous application of different levels of SA through the rooting medium improved the growth of both wheat cultivars under non-stress conditions, it did not mitigate the adverse effects of drought stress on growth of both cultivars. These results are contradictory to some earlier studies in which it has been observed that exogenous application of SA promotes the growth and counteracts the stress-induced growth inhibition due to abiotic stresses (water stress, salt stress, cold stress, heavy metal stress etc.) in different crop species (Metwally *et al.*, 2003; Shakirova *et al.*, 2003; Singh & Usha, 2003). In contrast, while working with maize, Németh *et al.*, (2002) reported that exogenously applied SA through the rooting medium caused an increase in growth inhibition. Likewise, Borsani *et al.*, (2001) demonstrated that transgenic *Arabidopsis* plants (*NahG*) producing salicylate hydroxylase, which transforms SA to catechol (thus lower concentration of endogenous SA in transgenic plant than that in wild type plant), were better able to resist the oxidative damage generated by salt and osmotic stress than the wild type plants. These differences in growth responses to SA were possibly due to differences in level of SA applied. For example, in the present study, under non-stress conditions exogenous application of different levels (5 or 10 mg L<sup>-1</sup>) through the rooting medium improved the growth of both wheat cultivars. Likewise, the effective dose of SA applied through the rooting medium for improving growth of non-stressed plants of wheat was 0.05 mM (~7 mg L<sup>-1</sup>) (Bezrukova *et al.*, 2001). Similarly, 1 mM (138 mg L<sup>-1</sup>) SA for tomato (Tari *et al.*, 2002) and 0.5 mM (~70 mg L<sup>-1</sup>) for *Phaseolus vulgaris* (Stanton, 2004) were the effective doses in ameliorating salt induced adverse effects when applied through the rooting medium. However, root applied 0.5-1.0 mM (69-138 mg L<sup>-1</sup>) SA did not affect growth of soybean seedlings, whereas 5 mM (690 mg L<sup>-1</sup>) SA applied through rooting medium had adverse effects on growth of soybean seedlings (Lian *et al.*, 2000).

Photosynthesis is one of the most vital physiological processes contributing to plant growth and productivity of crops for food. In other words, photosynthetic capacity in crop plants is the primary component of dry matter productivity. However, rate of photosynthesis varies with the change in environmental factors thereby affecting plant growth and yield (Nátr & Lawlor, 2005). In the present study, exogenously applied SA through the rooting medium caused an increase in photosynthetic rate under non-stress or drought stress conditions. This increase in photosynthetic rate due to exogenously applied

SA through the rooting medium was in agreement with some earlier studies in which it was found that exogenously applied SA increased the photosynthetic rate in different crops, e.g., soybean (Khan *et al.*, 2003), wheat (Singh & Usha, 2003), and maize (Khan *et al.*, 2003; Khodary, 2004). However, this increasing effect was more pronounced only in non-stressed plants grown in short-term hydroponics at 5 or 10 mg L<sup>-1</sup> SA applied through the rooting medium. Salicylic acid-induced increase in photosynthesis can be associated with stomatal or non-stomatal factors (Athar & Ashraf, 2005). In the present study, stomatal conductance ( $g_s$ ) and transpiration rate ( $E$ ) were increased with increase in photosynthetic rate ( $A$ ) due to exogenously applied SA through rooting medium, which suggests that SA-induced increase in photosynthesis might have been due to stomatal factors.

All macronutrients (N, P, K<sup>+</sup>, and Ca<sup>2+</sup>) are essentially required for the activities of enzymes, protein synthesis, integrity of cell wall and plasma membrane, and as components of proteins, photosynthetic protein complexes, photosynthetic pigments, RNA and DNA (Taiz & Zeiger, 2002). The results for macronutrients (N, P, K<sup>+</sup> and Ca<sup>2+</sup>) analyzed in the present study clearly indicate that water stress decreased all macronutrients in the shoot. However, exogenously applied SA did not alter all these macronutrients except root K<sup>+</sup> contents both under control and water stress conditions. A sharp decrease in root K<sup>+</sup> contents of non-stressed and water stressed plants due to application of SA through the rooting medium as observed in the present experiment was also supported by other reports like substantial reduction of potassium absorption in barley roots due to salicylic acid application @ 0.05 mM (Glass, 1974). Salicylic acid also inhibited potassium absorption in oat roots in a pH and concentration dependent manner (Harper & Balke, 1981). Contrastingly, El-Tayeb (2005) observed that the potassium contents decreased in case of shoots of the control and salt stressed plants with salicylic acid application while it was increased in plant roots. A slight improvement in the shoot Ca<sup>2+</sup> contents due to SA application observed in cv. S-24 under water deficit conditions is similar to the findings of Kawano and Muto (2000) where SA caused an increase in cytosolic Ca<sup>2+</sup> concentration in tobacco cell suspension culture. An increase in cytosolic Ca<sup>2+</sup>, as a second messenger, might induce further physiological responses including expression of osmotic responsive genes (Pardo *et al.*, 1998).

From the above discussion, it can be concluded that exogenously applied SA through the rooting medium caused an increase in growth of both cultivars under non-stress conditions. Although exogenous application of SA rooting medium caused changes in gas exchange characteristics of both cultivars, they could not induce drought tolerance. Thus, further studies are required to explicitly elucidate the mechanism of SA influx through different ways and the target enzymes or metabolites involved in plants to respond to SA application.

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